USE OF CONFOCAL LASER SCAN MICROSCOPY (CLSM) TO EVALUATE *S. epidermidis* BIOFILM STRUCTURE: THE EFFECT OF LOW CONCENTRATION OF ANTIBIOTICS– A CASE STUDY

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ABSTRACT

Staphylococcus epidermidis is the major organism causing nosocomial infections associated with the formation of a biofilm on medical devices. The major constituent of *S. epidermidis* biofilm matrix is the polysaccharide PNAG, synthesized by the proteins encoded in the *icaADBC* locus. CLSM low energy lasers allows the observation of living cells, and its penetration ability renders detailed tridimensional images of the biofilm. Another characteristic of CLSM associated with fluorochroms labeling is the ability to differentiate between biofilm cells and other constituents of the biofilm.
In this study, differences in the structure of a *S. epidermidis* biofilms grown in the presence or absence of low concentrations of dicloxacillin were assessed by CLSM observations of biofilms labeled with a fluorescent lectin (wheat germ agglutinin) that binds to PNAG.
Biofilms were formed in 6 wells polystyrene plates, during 48h at 37ºC and 120RPM in TSB supplemented with 1% of glucose in the presence and absence of sub-inhibitory concentration of dicloxacillin. The growth medium was carefully changed after 24h. CLSM observations were performed in a Zeiss LSM510 Meta microscope, using a single-channel analysis.
The results demonstrated that biofilms formed in the presence of dicloxacillin were less thick compared with the control. Furthermore biofilms formed in the presence of dicloxacillin produced lower amounts of PNAG.
The use of CLSM brought a new light into biofilm science by allowing the direct visualization of several processes that occurs inside the biofilm structure. In this particular case it was possible to demonstrate that low concentration of antibiotic alters the biofilm structure and composition.